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Extinction, Absorption, Scattering, and Backscatter for Aerosolized *Bacillus Subtilis* Var. *Niger* Endospores From 3 to 13 μm

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Computational and Information Sciences Directorate

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Abstract

Spectral extinction was measured in situ for aerosolized *Bacillus subtilis* var. *niger* (BG) endospores with the use of Fourier-transform infrared (FTIR) spectroscopy from 3.0 to 13.0 μm. Corresponding aerosol-size distributions were measured with the use of a commercially available elastic light-scattering probe and verified by direct particle capture and subsequent counting via video microscopy. Aerosol mass density was monitored simultaneously with conventional dosimetry and used to mass-normalize the measured spectral extinction. Mie theory calculations based on measured distributions and available complex indices of refraction agreed well. Also present are resultant Mie calculations for the absorption, total scattering, and backscattering. Included are the real and imaginary components of the complex index of refraction for BG as measured by Milham and Quarry. Both calculated and measured cross sections suggest that for wavelengths longer than 6.0 μm, the total extinction is primarily due to absorption. Finally, to offer a comparison, we present measured spectral extinction for three additional aerosols often found in the lower atmosphere, i.e., water fog, diesel soot, and Arizona road dust.

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1. Introduction

Infrared (IR) spectral extinction for common inorganic aerosols found in the lower atmosphere has been studied and reported on extensively [1–5]. However, research involving the optical properties for intact viruses, fungi, and bacterial aerosols is still extremely rare and woefully lacking [6–8]. Though much of the aggregate bioaerosol material in the lower atmosphere is mundane in nature, reported diseases in humans, animals, and plants have been linked to certain airborne bacteria [9–13]. Tong and Lighthart have reported ambient total atmospheric bacterial (TAB) concentrations for a rural environment that range from 0.1 to 0.001 airborne bacteria per cm^3 ($cells/cm^3$) [14,15].

The ability of one to detect the presence of a harmful bioaerosol from a safe distance is an ever-increasing topic of interest. Proposed optical methods that involve IR light are usually restricted to one or both of the atmospheric transmission window regions, i.e., 3 to 5 μm and/or 8 to 12 μm. These methods usually involve either passive or active illumination schemes, e.g., hyperspectral IR imaging or a CO_2 lidar-type arrangement [16–20]. Whether these (or other) optical techniques are appropriate for the remote detection and identification of harmful bioaerosols remains a topic of discussion. Nevertheless, for the utility of any particular approach to be properly evaluated, certain optical parameters at IR wavelengths for well-characterized bioaerosols are badly needed.

Methods for determining the electromagnetic interaction with bioaerosols usually involve either direct in situ light measurements or particles that exhibit some spherical symmetry (or that are Rayleigh); Mie or a comparable theory may be used to calculate the scattered and absorbed fields. For the latter approach in which the optical interaction is computed, the complex refractive indices for the biomaterials are assumed to be well known. Even when these refractive indices exist (which is rarely), accurate predictive calculations are usually extremely difficult to compute, since these types of particles are typically inhomogeneous and often nonspherical.

We directly measured the IR spectral extinction for aerosolized *Bacillus subtilis* var. *niger* (BG) endospores from 3 to 13 μm using conventional Fourier-transform infrared (FTIR) spectroscopy. Size distributions, aerosol densities, and particle morphology were measured simultaneously. We then compared these results with Mie theory calculations using complex indices of refraction provided by Milham and Quarry [20]. For regions in which there is good agreement between the measured and calculated extinction, we also present the total scatter, absorption, and backscatter components predicted by the Mie theory. Spectral extinction for both bulk powder and thin-film forms of BG is also presented. Finally, we contrast the extinction spectra for BG with three atmospheric aerosols commonly found in the environment, i.e., water fog, diesel soot, and Arizona road dust.

2. Experiment

The BG endospores used in this study were provided by the Edgewood Research Development and Engineering Center (ERDEC), Aberdeen Proving Ground, MD, and were produced in large quantities for use as a biological warfare (BW) simulant. This material, often referred to as "military-grade" BG, was assayed and known to contain 12.7×10^{10} colony-forming units per gram (cfu/g). Ion chromatography was performed on both washed and unwashed suspensions. Results showed small quantities of sulfate ions with lesser amounts of PO_4^{-3}, F^-, and Cl^- ions, which have been attributed to small quantities of residual growth media. Prior ultraviolet fluorescent studies have associated certain anomalous results to these sulfate ions [22]. IR extinction measured here for both washed and unwashed BG samples showed no appreciable differences between the spectra.

The primary transmission measurement was conducted in a 0.5-m^3 aerosol chamber, which provided an optical path length of 0.61 m. Dry and hydrated BG endospores in aerosol form were dispersed separately with a variety of techniques that have proven effective in prior studies. We generated hydrated endospore droplets using two pharmaceutical nebulizers that atomized various concentrations of a BG/water solution. To simulate conditions similar to the open atmosphere, we evaporated the encapsulating water droplets by directing the BG/water spray into a plenum of heated dry air. The resultant spore aerosol was gently drawn into the chamber with a small-area recirculating fan. We continuously monitored relative humidity using a filtered dew-point hygrometer that was inserted through the walls of the chamber.

Dry powdered BG was effectively aerosolized and sprayed into the chamber. Pressurized air was used to inject the endospore powder through a cylindrical nozzle that contained a spiraling array of fine stainless-steel wires. A vortex created within the nozzle effectively separated and dispersed the bacterial spores with minimal agglomeration. Care was taken to properly adjust the air pressure so that spore coatings remained reasonably intact.

We obtained IR transmission spectra using a high-resolution (0.02 wavenumber) Bomem DA2.02 FTIR spectrometer. For this study, the spectrometer was operated in a transmission mode, i.e., spectral attenuation was measured by placing the aerosol chamber between the source and the interferometer. A broadband IR Nernst glower was collimated with a ZnSe condensing lens assembly and projected through the aerosol chamber with two BaF_2 transmission windows that were fitted with dry-air flushes. Transmitted light was coupled to the interferometer with a gold-surfaced f/4 off-axis parabola.

Particle-size distributions were measured with a commercial particle-size spectrometer. We used Particle Measuring Systems, Inc. (PMS), particle spectrometer (model CSASP-100) to monitor in real time the aerosol size distribution. We determined particle-shaped characteristics by analyzing

photographs of captured particles that were collected on shielded glass slides. Using these photographs, we directly counted representative samples and generated a size distribution that agreed well with the distributions measured using the PMS. The resultant size distribution with a corresponding log-normal fit (r modal = 0.89 μm; standard deviation, $\sigma = 0.15$). A typical BG run is shown in figure 1. Photographs also showed that the endospore aerosol consisted of mostly single or minimally agglomerated particles that appeared reasonably spherical (see fig. 2). Aerosol mass densities (g/m^3) were periodically measured by collecting the aerosol on polycarbonate filters while sampling known volumes of air for predetermined periods of time. Results from the dosimetric sampling were then used to mass-normalize the measured extinction (m^2/g).

To offer a comparison, we measured the spectral extinction for three additional "background" aerosols commonly found in the environment, i.e., diesel soot, water fog, and Arizona road dust (SiO_2). Environmental aerosols were generated by either burning diesel fuel (soot), nebulizing distilled water (fog), or dispersing dry dust in a similar manner as just described (Arizona road dust). Spectra were measured and mass-normalized in the same fashion as the BG aerosol.

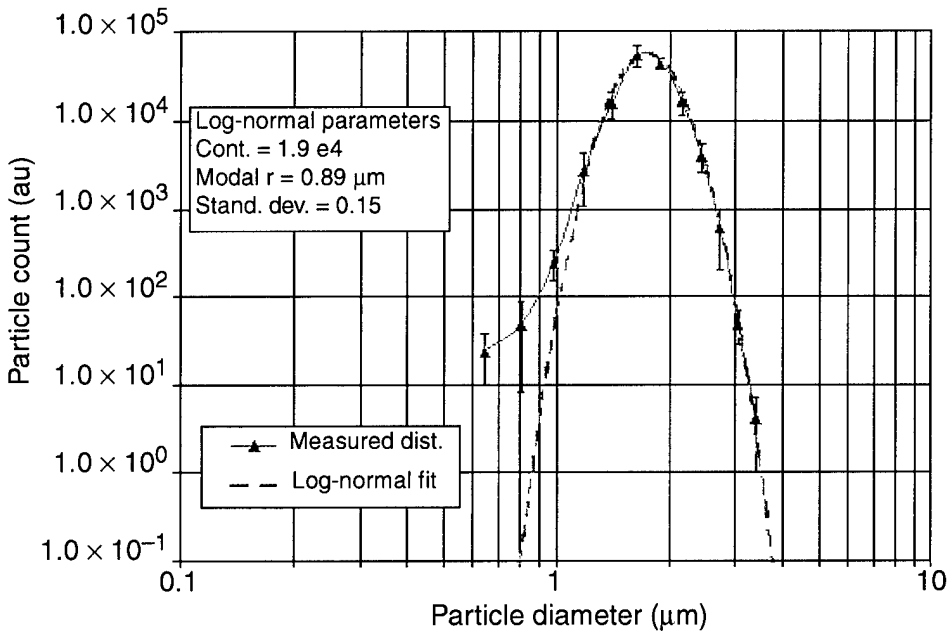
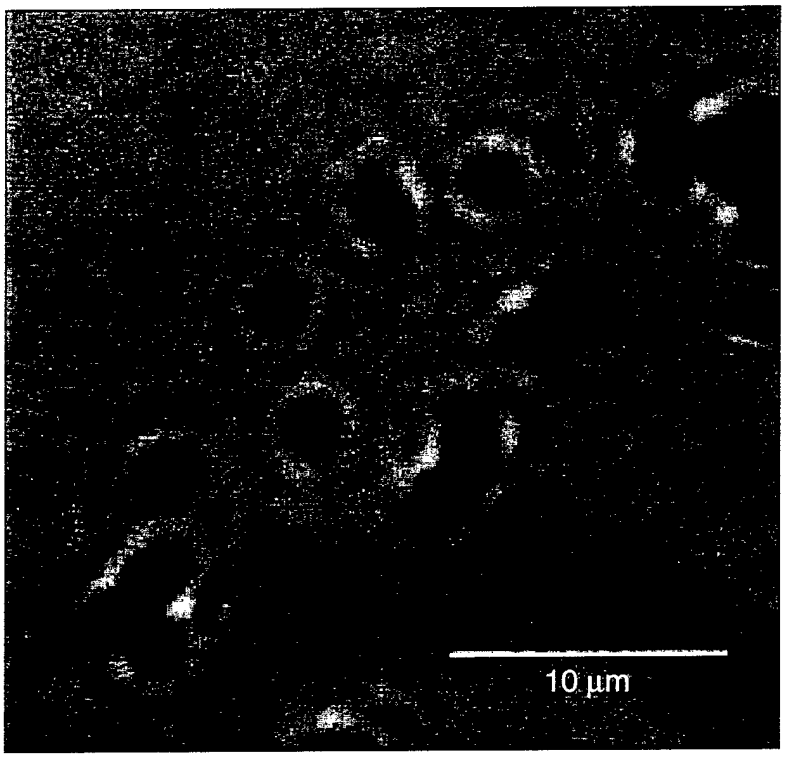


Figure 1. Measured BG size distribution with corresponding log-normal curve fit.

Figure 2. Single BG endospores shown here to be reasonably spherical.



3. Results

Interferograms were recorded before, during, and after each aerosol dispersion. We applied a Bartlet apodization to each interferogram before performing background rationing. Forward-scattering corrections were applied to the raw transmission and were found to be insignificant at the wavelengths above 6 μm. As the wavelength becomes comparable (or smaller) to the endospore diameter, the forward-scattering correction increases monotonically and was found to be as much as 9 percent at 3 μm [23]. Figure 3 shows a typical graph of the recorded transmittance from 3 to 13 μm for a series of concentrations of BG aerosol. Transmission was converted to extinction with a Beer's law relation. Results were mass-normalized by dividing the raw extinction (1/m) by the corresponding aerosol mass density (g/m^3) measured during the dosimetric portion of the experiment (see fig. 4).

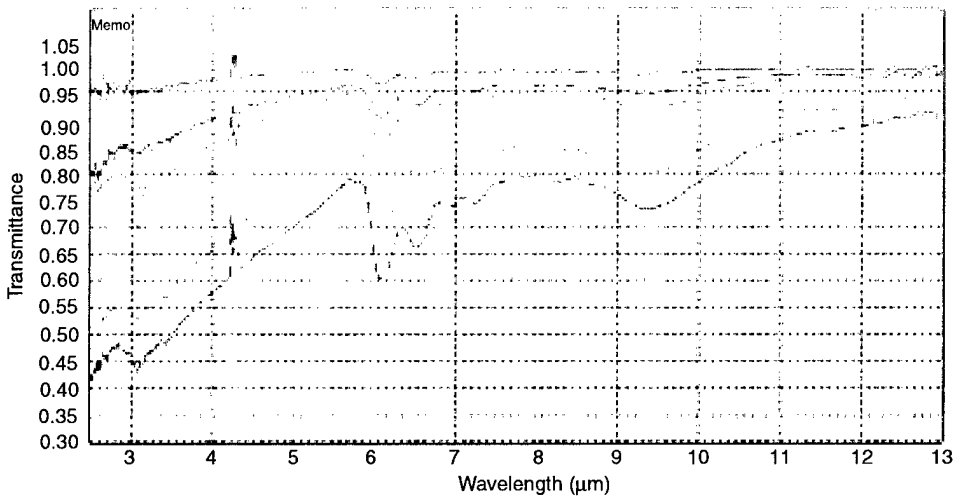


Figure 3. FTIR transmittance for various aerosol concentrations of BG.

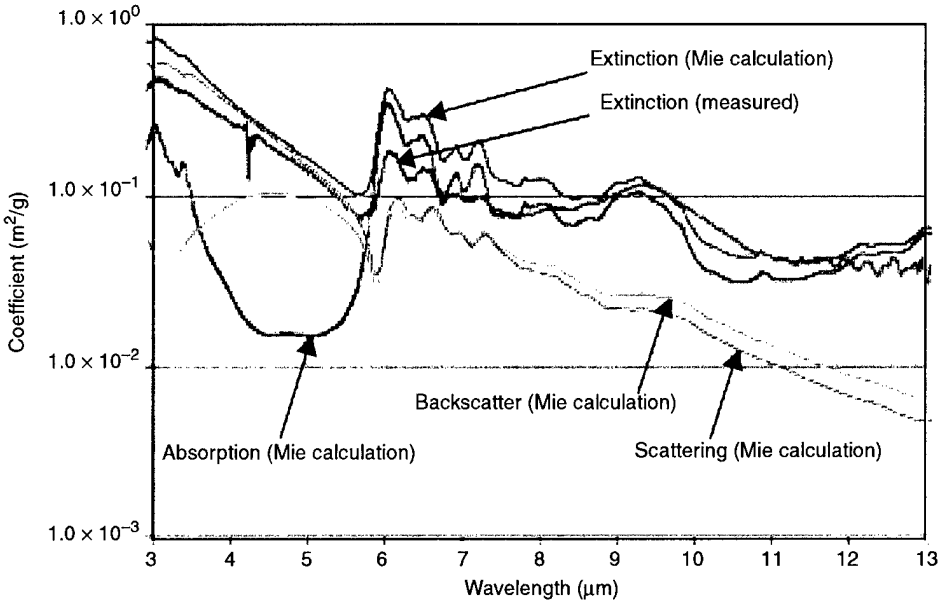


Figure 4. Measured and calculated mass-normalized (m^2/g) extinction, absorption, total scatter, and backscatter cross sections for aerosolized BG endospores.

Figure 4 shows both measured and calculated cross sections. Mie theory cross sections were calculated by convolving the measured size distribution with the complex indices of refraction (provided by Milham and Quarry) shown in figure 5 [20]. As one can see, the measured extinction shows similar form to the calculated spectra, albeit at slightly reduced levels. What is interesting and worth noting is that beyond 6 μm, the Mie calculations show that almost all the extinction for the BG aerosol is due to absorption.

Particle number densities were calculated by integrating the measured size distribution with the estimated mass per particle (bulk density for BG taken to be 1.45 g/cm^3) and equating it to the measured aerosol mass density [25]. Particle number densities for a typical run ranged from a low of 2×10^3 endospores/cm^3 for periods in which a significant amount of settling had occurred to peak values on the order of 1×10^6 endospores/cm^3 for periods shortly after the initial dispersion. Note that certain spectral features became difficult to resolve when particle number densities fell much below 10^3 endospores/cm^3 (based on our relatively small 0.61-m path length).

It is sometimes convenient to represent the coefficients shown in figure 4 on a "per-spore" basis. Using the measured size distribution and assuming the endospores and their agglomerates are reasonably spherical, we calculate a value of approximately 5×10^{-12} g/spore, which can then be used to convert the mass-normalized quantities shown in figure 4 (m^2/g) to cross sections per spore (m^2/spore). As an example, we find from figure 4 the mass-normalized extinction at 9.32 μm to be 1.16×10^{-1} m^2/g. Multiplying this value by the conversion factor, 5×10^{-12} g/spore, we calculate the extinction cross section per spore to be 0.583×10^{-12} m^2/spore. This compares well with the Mie theory calculated value of 0.525×10^{-12} m^2/spore.

To contrast these results, we measured the extinction spectra for three atmospheric aerosols, i.e., water fog, diesel soot, and Arizona road dust. A similar set of measurements was repeated for the three environmental aerosols. Care was taken to ensure that generated size distributions (especially for the water fog) were similar to those measured in the field [26]. Mass-normalized extinction spectra for the three aerosols are compared with BG and are shown in figure 6. Absorption caused by residual water vapor produced the fine structure seen between 5 and 8 μm in both the fog and soot spectra, and the apparent spike seen near 4.25 μm in all spectra (especially for soot) was identified as residual CO_2.

Figure 5. (a) Real and (b) imaginary indices of refraction for BG.

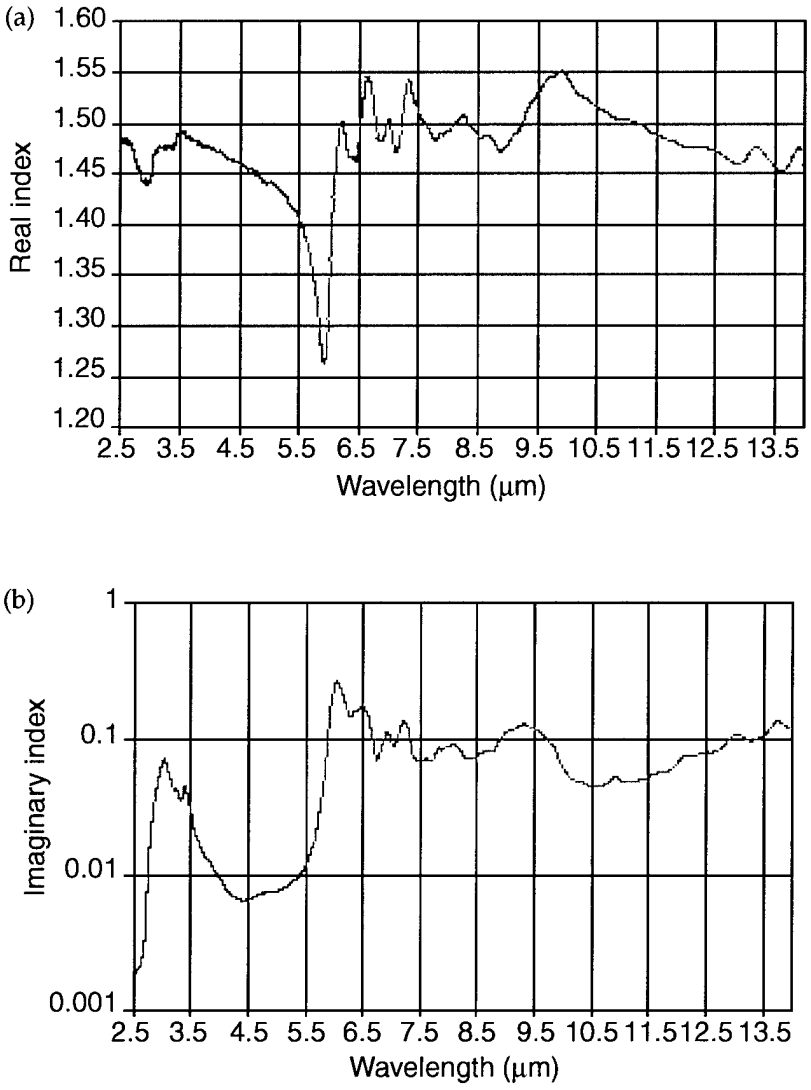
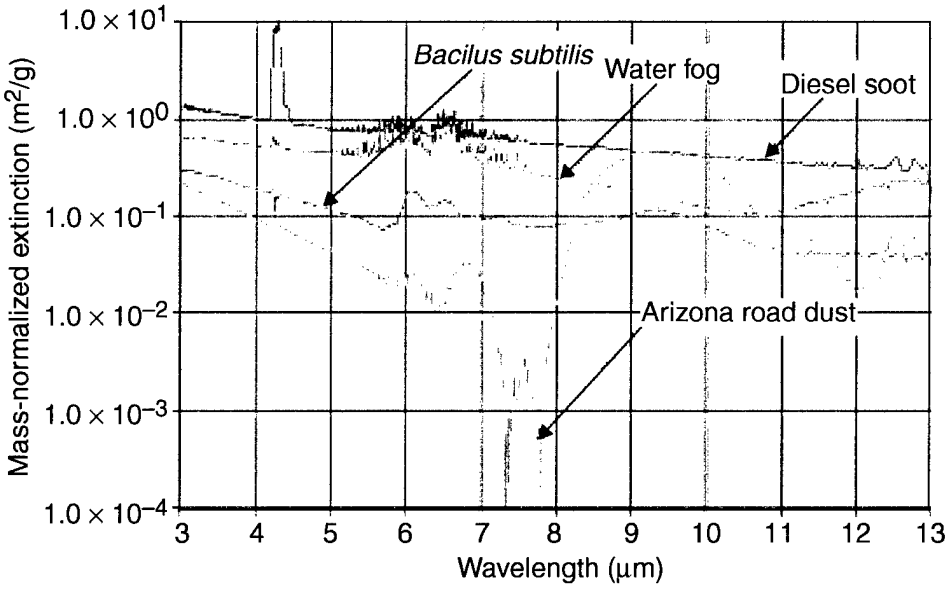


Figure 6. Comparison of measured mass-normalized extinction (m^2/g) for BG with three common background aerosols, i.e., water fog, Arizona road dust, and diesel soot.



4. Discussion

As seen in figure 6, BG shows relatively moderate extinction at the IR wavelengths considered (on a mass-normalized basis) when compared to the various background aerosols. As expected, the spectra for aerosolized BG are relatively smooth and devoid of any sharp identifiable "line" structure. While many of the IR bands of intact microorganisms still have to be assigned unambiguously, preliminary species identification conducted on biological thin-films has been reported [27].

The most characteristic features associated with the presence of certain spore proteins occur in the range between 5.6 and 6.6 μm and are attributed to the so-called "amide I and II" absorption bands. Unfortunately, this highly characteristic region lies in the middle of the most opaque portion of the atmosphere and is probably of little value when possible remote detection techniques are considered (i.e., because of strong water vapor absorption, transmission from 5 to 8 μm is nearly impossible) [28]. Naumann and Helm have tentatively identified the smooth broadly peaked region between 8 and 11 μm as a superposition of many fine absorption bands caused by C-O-C and C-O-P stretching of predominantly polysaccharide or phophodiester [29]. Naumann and Helm assert that the peak around 9.3 mm is due primarily to symmetric molecular vibrations of phosphate diesters. Beyond 11 μm, the region exhibits a variety of weak but extremely characteristic features attributed to aromatic ring vibrations of phenylalanine, tyrosine, tryptophan, and various nucleotides. With the exception of a few weak peaks around 13 μm (resulting from $>CH_2$ rocking modes of fatty acid chains), specific assignment is impossible. Although this region appears relatively featureless, Naumann and Helm have successfully used this 8- to 13-μm region for taxonomical identification of bacterial thin-films. Because of this, the region is often referred to as the "bacterial fingerprint region."

Because much of the existing information (albeit limited) involving IR spectra for biological materials is derived from thin-film samples, we thought it interesting to compare differences among extinction spectra for various forms of BG. Figure 7 shows a comparison of the spectral extinction for aerosolized BG, bulk powdered BG (measured with attenuated total reflection (ATR)), and thin-film spectra taken from Yabushita and Wada [27]. As expected, much of the spectral information is similar from one form to another for regions where absorption is predicted to dominate. Also seen in figure 7 are the effects caused by scattering. As the ratio of the spore diameter to wavelength becomes larger, scattering begins to dominate in the aerosol extinction. Certain absorption features seen near 3 μm for the bulk and thin-film spectra are completely obscured by particulate scattering and are not seen in the aerosol spectra.

As one can see in figures 4, 6, and 7, most aerosol spectra tend to be devoid of any easily identifiable characteristic features. This makes reliable identification difficult with the use of conventional spectra correlation techniques. Naumann and Helm have demonstrated the ability to rapidly classify and

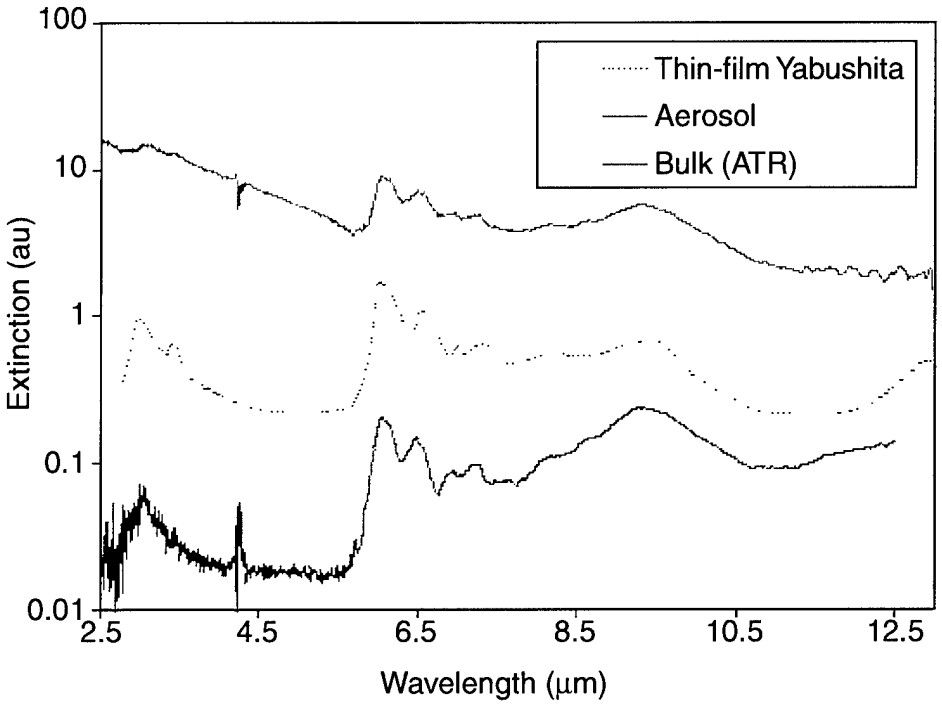


Figure 7. Comparison of FTIR measurements conducted on various forms of BG, i.e., aerosolized BG (top), thin-film slurry (middle), and bulk powdered BG (bottom).

group intact bacterial specimens using a technique called "cluster analysis" [6]. Their approach is based on treating spectra as images or patterns and applying classic pattern recognition algorithms to discriminate subtle differences among similar spectra. A variety of simple mathematical operations is applied to weighted wavelength regions based on certain predetermined criteria. In addition, when comparing one spectral form to another, one must consider carefully how the aerosol spectra were recorded. Bryson and Flanigan have reported on an obvious but interesting effect witnessed during a series of FTIR aerosol field measurements [28]. They report that for wavelengths in which aerosols are highly absorbing, as is for BG above 6 μm, measured transmission may appear as either an extinction or *emission* spectra (one being the mirror image of the other), depending on whether the background is considered hot or cold relative to the aerosol. The symmetry relation between absorption and emission spectra is a direct result of Kirchhoff's law that relates aerosol absorption efficiencies to particle emissivity [29]. Under such conditions, it would be desirable to consider a "form"-preserving operation that is independent of aerosol density and/or background conditions.

As an example, we apply two simple form-preserving operations on the measured extinction spectra, i.e., first and second derivatives (see fig. 8). Figure 8 shows the original extinction for each aerosol measured at various concentrations (top spectra in each frame). Below each extinction curve, we show the resultant first and second derivatives. One fairly easy parameter to "key on" would be the spectral position of where the first and second derivatives cross zero (shaded narrow regions). Minor variance in defining these zeros arises when attenuation is extremely weak because of low aerosol concentrations.

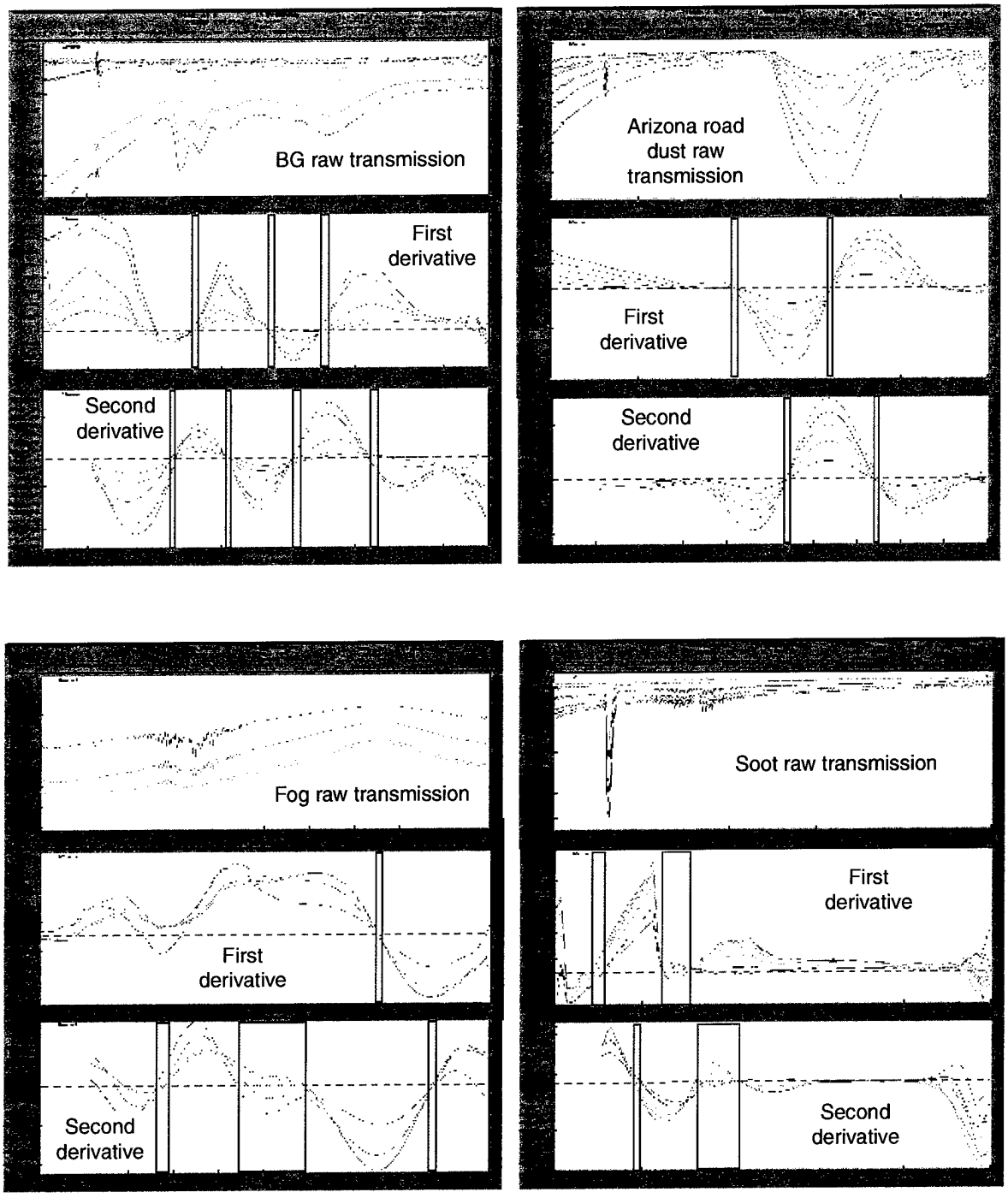


Figure 8. Example of two simple "form-preserving" operations (i.e., first and second derivatives) that one might consider to discriminate subtle differences among similar spectra. Each set of three frames consists of original transmission spectra measured for various aerosol densities (top) and corresponding first (middle) and second (bottom) derivatives.

The question remains: "Can one remotely identify the presence of a harmful bioaerosol using conventional IR transmission measurements like those conducted here?" The answer to this question is complex and a function of many parameters, e.g., optical depth, sensor response, ambient atmospheric conditions, etc. As a result, we make no attempt to give a definitive answer. However, certain key aspects should be addressed when considering such a scenario.

First, because aerosol extinction spectra have a tendency to be relatively smooth, low-resolution spectroscopy will usually suffice. Although the spectrometer used for this study was set at a resolving power of 4 cm^{-1}, no spectral features were observed that could not be resolved at a substantially lower setting, for example, 20 cm^{-1}. Second, a reasonable amount of bioaerosol material must obviously be present (relative to ambient conditions) to discern noticeable changes in the transmission.

To get an "order of magnitude" estimate of the bioaerosol concentrations necessary to be spectroscopically measurable, let us first assume a simple transmisometer-type arrangement in which a homogenous bioaerosol cloud obscures a sufficiently powerful broadband IR source. Assuming an endospore cloud 100 m in extent obscures a similarly dimensioned optical path for a collimated beam (diameter 4 cm), we estimate a minimum detectable particle density (to achieve a reasonable amount of attenuation) to be on the order of 10 to 20 particles/cm^3.

Up to this point, we have assumed that clear conditions and effects caused by the intervening atmosphere were negligible, i.e., spectral masking because of path radiance and molecular absorption by gaseous CO_2, H_2O, and O_3. When these effects are considered and the distance from the spectrometer to the biocloud is in excess of 5 km or more, the minimum detection limits stated in the previous paragraph increase several fold. This seems to restrict the type of approach one might consider to the "plume identification" realm in which the measurement is conducted near to the source and that the bioaerosol cloud is sufficiently dense. Based on this and our experience in measuring aerosol spectra, we believe that the remote detection of a bioaerosol using IR extinction spectra will be limited to active illumination techniques in which the source of radiation is sufficiently strong enough to overcome the detrimental effects caused by atmospheric absorption and path/ground radiance.

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Spectral extinction was measured in situ for aerosolized *Bacillus subtilis* var. *niger* (BG) endospores with the use of Fourier-transform infrared (FTIR) spectroscopy from 3.0 to 13.0 μm. Corresponding aerosol-size distributions were measured with the use of a commercially available elastic light-scattering probe and verified by direct particle capture and subsequent counting via video microscopy. Aerosol mass density was monitored simultaneously with conventional dosimetry and used to mass-normalize the measured spectral extinction. Mie theory calculations based on measured distributions and available complex indices of refraction agreed well. Also present are resultant Mie calculations for the absorption, total scattering, and backscattering. Included are the real and imaginary components of the complex index of refraction for BG as measured by Milham and Quarry. Both calculated and measured cross sections suggest that for wavelengths longer than 6.0 μm, the total extinction is primarily due to absorption. Finally, to offer a comparison, we present measured spectral extinction for three additional aerosols often found in the lower atmosphere, i.e., water fog, diesel soot, and Arizona road dust.

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